

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1-39. (cancelled)

40. (currently amended) A method for preparing a specific recombinant protein, said method being carried out by overexpression of a gene encoding for [[a]] the specific recombinant protein in a monokaryotic strain of filamentous fungi of the species *Pycnoporus* of the basidiomycete group, comprising:

(a) ~~a stage of~~ culturing the ~~abovementioned~~ monokaryotic strain of *Pycnoporus*, said strain being transformed ~~using~~ with an expression vector containing a gene encoding for [[a]] the specific recombinant protein, the expression of ~~which~~ ~~is~~ said gene being placed under the control of pLac3 promoter represented by SEQ ID ON: 3,

~~a promoter corresponding to an endogenous promoter of the abovementioned fungi, or of a exogenous promoter), said promoter being constitutive or inducible,~~

(b) ~~a stage of induction of~~ inducing the ~~abovementioned~~ pLac3 promoter, ~~when the latter is inducible,~~ and

(c) recovering and purifying ~~of~~ the specific recombinant protein, produced in the culture medium.

41. (currently amended) The method according to claim 40, wherein the monokaryotic strain of *Pycnoporus* ~~used~~ is a strain of *Pycnoporus cinnabarinus*.

42. (currently amended) The method according to claim 40, wherein the specific recombinant proteins overexpressed correspond to endogenous proteins of *Pycnoporus*, or to exogenous ~~proteinse~~ proteins corresponding to endogenous proteins of strains of *Pycnoporus* different from the strain of *Pycnoporus* used for the production of said proteins.

43. (currently amended) The method according to claim 40, wherein the specific recombinant proteins correspond:

(I) ~~(a)~~ to the following endogenous proteins of *Pycnoporus*:

- (i) the metalloenzymes, or
- (ii) cellobiose dehydrogenase, xylanase, β -glycosidase, invertase, or α -amylase, or

(II) ~~(b)~~ to the exogenous proteins selected from the group consisting of:

- (i) tyrosinases of strains of *Pycnoporus* different from the strain of *Pycnoporus* used for the production of said proteins,

(ii) laccases of basidiomycetes other than *Pycnoporus*, and

(iii) cinnamoyl esterases A and B of *Aspergillus niger*.

44. (currently amended) The method according to claim 40, wherein

specific recombinant proteins corresponding to the endogenous proteins of *Pycnoporus* are prepared, and

the monokaryotic strain of *Pycnoporus* ~~used~~ is deficient in the gene encoding for the endogenous protein to which the specific recombinant protein corresponds.

45. (currently amended) The method according to claim 40, wherein,

specific recombinant proteins corresponding to the endogenous proteins of *Pycnoporus* are prepared, and

the monokaryotic strain of *Pycnoporus* ~~used~~ is transformed ~~using~~ with an expression vector containing the gene encoding for the specific recombinant protein labelled ~~in particular~~ by a histidine label.

46. (currently amended) The method according to claim 40, wherein, recombinant laccases corresponding to the endogenous

laccases of *Pycnoporus* are prepared, ~~and~~ the method ~~comprises~~
comprising:

(a) ~~a stage of~~ culturing a monokaryotic strain of
Pycnoporus deficient in the gene encoding for the endogenous
laccase of *Pycnoporus* transformed ~~using~~ with an expression vector
containing the gene encoding for a laccase of *Pycnoporus*, ~~and~~ the
expression of ~~which is placed~~ said gene being under the control
of [[a]] said pLac promoter ~~corresponding to the endogenous~~
~~promoter of this laccase,~~

(b) ~~a stage of induction of~~ inducing the ~~abovementioned~~
pLac promoter, ~~in particular~~ by adding ethanol, or agricultural
by-products containing lignocellulose or compounds with an
aromatic ring, and

(c) recovering and purifying ~~of~~ the recombinant
laccase, corresponding to the ~~abovementioned~~ endogenous laccase
of *Pycnoporus* produced in the culture medium.

47. (currently amended) The method according to claim
46, wherein, recombinant ~~laccase~~ laccases corresponding to the
endogenous laccase of *Pycnoporus cinnabarinus* represented by SEQ
ID NO: 2 are prepared, ~~and~~ the method ~~comprises~~ comprising:

(a) ~~a stage of~~ culturing a monokaryotic strain of
Pycnoporus cinnabarinus deficient in the gene encoding for the
endogenous laccase of *Pycnoporus cinnabarinus*, transformed ~~using~~
with an expression vector containing the nucleotide sequence SEQ

ID NO: 1 encoding for the recombinant laccase represented by SEQ ID NO: 2, ~~and the expression of which is said laccase being placed under the control of the plae promoter corresponding to the endogenous promoter of the abovementioned laccase, the sequence of said plae pLac3 promoter being represented by SEQ ID NO: 3,~~

(b) ~~a stage of induction~~ inducing by ethanol ~~of the abovementioned plae pLac3 promoter, and~~

(c) ~~the recovery, and the purification of~~ recovering and purifying the recombinant laccase, represented by SEQ ID NO: 2 produced in the culture medium.

48-49. (cancelled)

50. (currently amended) The method according to claim 40, wherein recombinant tyrosinase corresponding to the tyrosinase of *Pycnoporus sanguineus* represented by SEQ ID NO: 16 ~~are is~~ prepared, ~~and the method comprises~~ comprising:

(a) ~~a stage of~~ culturing a monokaryotic strain of *Pycnoporus cinnabarinus* transformed using an expression vector containing the nucleotide sequence SEQ ID NO: 15 encoding for the recombinant tyrosinase represented by SEQ ID NO: 16, the sequence SEQ ID NO: 15 being advantageously preceded by the a nucleotide sequence encoding the first 21 amino acids, which is ~~the a~~ peptide signal, of SEQ ID NO: 2, ~~and the expression of which is~~

said tyrosinase being placed under the control of the ~~plac~~ pLac3 promoter corresponding to the endogenous promoter of the laccase of *Pycnoporus cinnabarinus*, the sequence of said ~~plac~~ pLac3 promoter being represented by SEQ ID NO: 3,

(b) ~~a stage of induction~~ inducing by ethanol of the ~~abovementioned plac~~ pLac3 promoter, and

(c) recovering and purifying the recombinant tyrosinase, represented by SEQ ID NO: 16 produced in the culture medium.

51. (currently amended) The method according to claim 40, wherein recombinant laccase corresponding to the laccase of *halocyphina villosa* represented by the sequence SEQ ID NO: 18 is prepared, ~~and the method comprises~~ comprising:

(a) ~~a stage of~~ culturing a monokaryotic strain of *Pycnoporus cinnabarinus* deficient in the gene encoding for the endogenous laccase of *Pycnoporus cinnabarinus*, transformed using an expression vector containing the nucleotide sequence represented by the sequence SEQ ID NO: 17[[+]] encoding for the recombinant laccase represented by SEQ ID NO: 18, ~~and the expression of which is~~ said laccase being placed under the control of the ~~plac~~ pLac3 promoter corresponding to the endogenous promoter of the laccase of *Pycnoporus cinnabarinus*, the sequence of said ~~plac~~ pLac3 promoter being represented by SEQ ID NO: 3,

(b) ~~a stage of induction~~ inducing by ethanol of the ~~abovementioned pLac~~ pLac3 promoter, and

(c) recovering and purifying of the recombinant laccase, represented by SEQ ID NO: 18 produced in the culture medium.

52. (withdrawn) Nucleotide sequence encoding for the pLac promoter of the endogenous laccase of *Pycnoporus cinnabarinus*, and corresponding to the sequence SEQ ID NO: 3, or any sequence derived from this promoter by substitution, addition or suppression of one or more nucleotides and retaining the property of being a promoter of the expression of sequences.

53. (withdrawn) Expression vector characterized in that it comprises the sequence SEQ ID NO: 3 encoding for the pLac promoter of the endogenous laccase of *Pycnoporus cinnabarinus*.

54 (withdrawn) Expression vector according to claim 53, characterized in that it comprises a gene encoding for a specific recombinant protein, and the expression of which is placed under the control of the pLac promoter.

55. (withdrawn) Expression vector according to claim 54, characterized in that the specific recombinant protein is a protein corresponding: to the following endogenous proteins of

Pycnoporus: the metalloenzymes, such as laccase, or tyrosinase, or cellobiose dehydrogenase, xylanase, .beta.-glycosidase, invertase, or .alpha.-amylase, to the exogenous proteins chosen from the following: the tyrosinases of strains of *Pycnoporus* different from the strain of *Pycnoporus* used for the production of said proteins, such as the tyrosinase of *Pycnoporus sanguineus* when the strain of *Pycnoporus* used for the production of this tyrosinase is different from *Pycnoporus sanguineus*, the laccases of basidiomycetes other than *Pycnoporus*, such as the laccase of *halocyphina villosa* (*halophilic basidiomycete*), the cinnamoyl esterases A and B of *Aspergillus niger*.

56. (withdrawn) Host cell transformed using an expression vector according to claim 54.

57. (withdrawn) Host cell according to claim 56, corresponding to monokaryotic cells of strains of *Pycnoporus*, such as strains of *Pycnoporus cinnabarinus*.

58. (previously presented) The method according to claim 43, wherein the metalloenzymes are chosen from laccase or tyrosinase.

59. (previously presented) The method according to claim 43, wherein the tyrosinases of strains of *Pycnoporus*

different from the strain of *Pycnoporus* used for the production of said proteins, is the tyrosinase of *Pycnoporus sanguineus*.

60. (currently amended) The method according to claim 43, wherein the laccases of basidiomycetes other ~~that~~ than *pycnoporus* is the laccase of *halocyphina villosa*.

61. (previously presented) The method according to claim 46, wherein the lignocellulose is selected from the group consisting of wheat straw, corn bran and beet pulp.

62. (previously presented) The method according to claim 46, wherein the compounds with an aromatic ring is selected from the group consisting of 2,5-xylidine, veratrylic acid, guaicol, veratrylic alcohol, syringaldazine, ferulic acid, caffeic acid and the lignosulphonates.

63. (new) The method according to claim 40, for preparing recombinant laccases corresponding to the endogenous laccases of *Pycnoporus*, the method comprising:

(a) culturing a monokaryotic strain of *Pycnoporus* transformed with an expression vector containing a gene encoding for a laccase of *Pycnoporus*, the expression of said gene being under the control of the pLac3 promoter,

(b) inducing the pLac3 promoter by adding ethanol, or agricultural by-products containing lignocellulose, or compounds with an aromatic ring, and

(c) recovering and purifying the recombinant laccases corresponding to the endogenous laccase of *Pycnoporus* produced in the culture medium.

64. (new) The method according to claim 46, for preparing the recombinant laccases corresponding to endogenous laccases of *Pycnoporus cinnabarinus* represented by SEQ ID NO: 2, the method comprising:

(a) culturing a monokaryotic strain of *Pycnoporus cinnabarinus*, transformed using an expression vector containing the nucleotide sequence SEQ ID NO: 1 encoding the recombinant laccase represented by SEQ ID NO: 2, the expression of said recombinant laccase being under the control of the pLac3 promoter corresponding to the endogenous promoter of the laccase, the sentence of said pLac3 promoter being represented by SEQ ID NO: 3,

(b) inducing by ethanol the pLac3 promoter,

(c) recovering and purifying the recombinant laccase, represented by SEQ ID NO: 2 produced in the culture medium.

65. (new) The method according to claim 40, for preparing recombinant laccases corresponding to the laccase of

halocyphina villosa represented by the sequence SEQ ID NO: 18,
the method comprising:

(a) culturing a monokaryotic strain of *Pycnoporus cinnabarinus* transformed using an expression vector containing the nucleotide sequence represented by the sequence SEQ ID NO: 17 encoding for the recombinant laccase represented by SEQ ID NO: 18, the expression of said laccase being placed under the control of the pLac3 promoter corresponding to the endogenous promoter of the laccase of *Pycnoporus cinnabarinus*, the sequence of said pLac3 promoter being represented by SEQ ID NO: 3,

(b) inducing by ethanol the pLac3 promoter,

(c) recovering and purifying the recombinant laccase,
represented by SEQ ID NO: 18 produced in the culture medium.